Abstract No. Chan0178

Structural Studies of Prokaryotic RNA Polymerase

S. Darst, E. Campbell, O. Muzzin, K. Murakami (Rockefeller U.)

Beamline(s): X9A

Transcription is the major control point of gene expression and RNA polymerase (RNAP) is the central enzyme of transcription. Our structural studies focus on bacterial RNAPs because of the high degree of conservation of RNAP structure and function from bacteria to man. We have performed work on X9A in connection with our studies of the promoter-specificity sigma subunit, which is the key regulator of bacterial transcription.Proteolysis of Thermus aquaticus SigA, which occurred in situ during crystallization, revealed three domains, Sig2, Sig3, and Sig4, connected by flexible linkers. Crystal structures of each domain were determined, as well as of Sig4 complexed with –35 element DNA (Sig2-Sig3 fragment, 2.9 Å resolution; Sig4, 1.8 Å resolution; Sig4/DNA, 2.4 Å resolution). Each structure was solved by SeMet MAD experiments using data collected at X9A. Exposed surfaces of each domain are important for RNA polymerase binding. Universally conserved residues important for –10 element recognition and melting lie on one face of Sig2, while residues important for extended –10 recognition lie on Sig3. Genetic studies correctly predicted that a helix-turn-helix motif in Sig4 recognizes the –35 element, but not the details of the protein-DNA interactions. Positive control mutants in Sig4 cluster in two regions, positioned to interact with activators bound just upstream or downstream of the –35 element. We also frequently use X9A to screen our RNAP crystals for subsequent use at insertion device beamlines, which is an extremely important application for us.